These amendments were made to more particularly point out and distinctly claim the subject matter that the Applicants regard as their invention.

REJECTION UNDER 35 U.S.C. §112(2)

Claims 33-46 were rejected as allegedly vague and indefinite. Claims 33-35 and 37-41 were rejected because it was unclear whether the recitation of the specific amino acid mutations in these claims referred to the heavy chain or the light chain. However, because the two chains are expressed as a single "holotoxin" (subsequently cleaved within the bacterial cell to form the two chains) it is conventional in the art to number the two chains of the toxin as they are originally found in the single chain. Thus, the recitation of a specific amino acid by number in the claims unambiguously indicates in which chain the amino acid resides.

Also, the Examiner rejected as vague the language "botulinum toxin other than A with a modification at a site corresponding to" a specific amino acid residue in toxin A. Applicants respectfully submit that the person of ordinary skill in the art would recognize that in addition to subtype A, there are also botulinum toxin subtypes B-G which have similar but non-identical amino acid sequences (see specification, page 3, lines 24-26). Thus, a subtype site "corresponding to a site in toxin A" would be understood to mean the site in the non-A toxin occurring at the same position in an amino acid sequence alignment as the identified site in botulinum toxin A.

Finally, the Examiner required the replacement of the terms SNAP-25 and VAMP in claims 39 and 44 with the full terminology. These claims have been amended to comply with the Examiner's requirement, and two additional proteins, syntaxin and cellubrevin have been added to Markush group of the claim. As stated above, these additions are supported at page 1 of the specification.

For these reasons, Applicants submit that the claims are now in compliance with 35 U.S.C. §112(2).

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REJECTION UNDER 35 U.S.C. §103(a)

Claims 33, 34, 36-38, 40, 42, 43 and 45 were rejected as obvious over Bizzini 4,594,336 in view of Fraenkel-Conrat. According to the Examiner, Bizzini teaches the use of a composition that comprises tetanus toxin that can be used to transport agents to the CNS, while Fraenkel-Conrat teaches mutation of the tetanus toxin light chain to destroy toxicity. Applicants respectfully traverse this ground of rejection, and submit that the combination of these references fail to suggest the present invention for at least two reasons.

First, the composition of Bizzini does not actually comprise the tetanus toxin, but rather comprises an atoxic fragment (of unknown amino acid sequence) thereof termed fragment B-IIb. Column 2, line 50-51 of the Bizzini '336 patent refers to a publication which describes this fragment in greater detail.

Applicants have acquired this reference (Bizzini et al., *J. Neurochem.* 28:529-542 (1977)) (hereinafter the "Bizzini Paper") , and now submit a copy thereof with this Reply in an accompanying Information Disclosure Statement and Form 1449 for the Examiner's convenience. This reference describes that fragment B-IIb is obtained from frozen concentrated filtrates of cultures of *C. tetani* which had apparently been subject to autoproteolysis. See Bizzini Paper, at paragraph bridging pages 529 and 530 and following paragraph. Fragment B-IIb was prepared by ammonium sulfate fractionation and subsequent serial gel filtration to yield a polypeptide preparation of apparent molecular weight 35,000 Da. See Bizzini Paper at 530, 4th full paragraph. By contrast, the two-chain native tetanus toxin and the botulinum toxins all have a molecular weight of about 150,000 Da.

This latter point is important because the instant specification makes clear that the Applicants discovered that both the heavy and light chains of the Clostridial neurotoxins are required for optimal receptor-ligand binding, see specification at page 6, lines 20-21. While not wishing to be bound by theory, this is thought to be because the receptor recognizes the quaternary structure of the dichain toxin. The present claims clearly indicate that the modified toxin of the instant invention comprises a light chain

inactivated by mutation and an unaltered heavy chain. Thus, the Bizzini '336 patent in no way suggests the surprising advantage of having both chains present in the transport proteins of the present invention.

It is true that the Bizzini Paper indicates that fragment B-IIb binds to insolubilized gangliosides with high affinity in vitro. However, the natural cell surface receptor for tetanus toxin is a protein rather than merely a sugar, and those of skill in this field recognize that the ability to bind gangliosides is largely irrelevant to the ability of a Clostridial neurotoxin to optimally bind its cell surface receptor.

To accentuate the second ground of distinction between the present invention and the cited references, the present claims have been amended to indicate that the drug or bioactive molecule is joined to the inactivated light chain. Support for this amendment can be found in the specification at page 14, lines 29. In this way, the drug is attached to the toxin moiety that actually enters the cytoplasm of the target neural cell by translocation through the endosomal membrane after receptor binding and endocytosis.

By contrast, the Bizzini '336 patent discloses attachment of a drug to the B-IIb fragment by thiolation generally without regard to the location of the drug with respect to the fragment. See generally, Bizzini '336, columns 5-7. Thus, the Bizzini transport molecule appears to potentially comprise a collection of B-IIb-based transport proteins having different points of drug attachment and different amounts of drug attached thereto. While such a preparation may retain the ability to deliver drug to the cytoplasm of the target cell, it lacks the advantages of the present invention with regard to efficiency of directed drug "loading" and raises potential reproducablity problems.

With these considerations in mind, the combination of Fraenkel-Conrat to Bizzini '336 can be seen to in no way suggest the invention of the present claims, wherein the modified toxin comprises both mutated light chain and unaltered heavy chain, and wherein the drug is joined to the light chain. Fraenkel-Conrat merely adds the fact the light chain can be inactivated by mutation and that the reconstituted mutant toxin is atoxic. There is no suggestion in the combination of references that it is important for transporter-receptor binding for both heavy and light chain to be present. And even assuming *arguendo* that the combination of references would lead one to attempt to use